

WHAT IS CLAIMED IS:

1. A method for preparing a G-CSF analog comprising the steps of:

- 5 (a) viewing information conveying the three dimensional structure of a G-CSF molecule;
- (b) selecting from said viewed information at least one site on said G-CSF molecule for alteration;
- 10 (c) preparing a G-CSF molecule having such alteration; and
- (d) optionally, testing such G-CSF molecule for a desired characteristic.

2. A computer based method for preparing a G-CSF analog comprising the steps of:

- 15 (a) providing computer expression of the three dimensional structure of a G-CSF molecule;
- (b) selecting from said computer expression at least one site on said G-CSF molecule for alteration;
- 20 (c) preparing a G-CSF molecule having such alteration; and,
- (d) optionally, testing such G-CSF molecule for a desired characteristic.

25 3. A method for preparing a G-CSF analog with the aid of a computer comprising:

- (a) providing said computer with the means for displaying the three dimensional structure of a G-CSF molecule including displaying the composition of moieties of said G-CSF molecule, preferably
- 30 displaying the three dimensional location of each amino acid, and more preferably displaying the three dimensional location of each atom of a G-CSF molecule;
- (b) viewing said display;

(c) selecting a site on said display for alteration in the composition of said molecule or the location of a moiety; and

(d) preparing a G-CSF analog with such
5 alteration.

4. A computer-based method for preparing a G-CSF analog comprising the steps of:

(a) viewing the three dimensional structure of a G-CSF molecule via a computer, said
10 computer having been previously programmed (i) to express the coordinates of a G-CSF molecule in three dimensional space, and (ii) to allow for entry of information for alteration of said G-CSF expression and viewing thereof;

15 (b) selecting a site on said visual image of said G-CSF molecule for alteration;

(c) entering information for said alteration on said computer;

(d) viewing a three dimensional
20 structure of said altered G-CSF molecule via said computer;

(e) optionally repeating steps (a)-(e) above;

(f) preparing a G-CSF analog with said
25 alteration; and

(g) optionally testing said G-CSF analog for a desired characteristic.

5. In a computer-based apparatus for displaying the three dimensional structure of a
30 molecule, the improvement comprising means for correlating said three dimensional structure of a G-CSF molecule with the composition of said G-CSF molecule.

6. A method for crystallization of a protein comprising the steps of:

35 (a) combining, optionally by automated means, aqueous aliquots of said protein with either (i)

aliquots of a salt solution, each aliquot having a different concentration of salt; or (ii) aliquots of a precipitant solution, each aliquot having a different concentration of precipitant;

5 (b) selecting at least one of said combined aliquots, said selection based on the formation of precrystalline forms, or, if no precrystalline forms are so produced, increasing the protein starting concentration of said aqueous aliquots
10 of protein and repeating step (a);

(c) after said salt or said precipitant concentration is selected, repeating step (a) with said previously unselected solution in the presence of said selected concentration; and,

15 (d) repeating step (b) and step (a) until a crystal of desired quality is obtained.

7. A method of claim 6 wherein each combination pursuant to step (a) is performed in a range of pH.

20 8. A method of claim 6 wherein said combining of step (a) is done in the presence of a nucleation initiation unit.

9. A G-CSF analog having an amino acid sequence different from that of Figure 1 in that:

25 (a) the N-terminal methionine is optional; and

(b) one or more of amino acids 58-72 (i) is substituted with one or more different amino acids or (ii) deleted; or (iii) chemically modified.

30 10. A G-CSF analog of claim 9 wherein said analog is more resistant to proteolysis than a G-CSF molecule of Figure 1.

11. A G-CSF analog of claim 10 wherein at least one of said amino acids is chemically modified by
35 the addition of a polyethylene glycol molecule.

12. A G-CSF analog having an amino acid sequence different from that of Figure 1 in that:

(a) the N-terminal methionine is optional; and

5 (b) one or more of amino acids 119-125 (i) is substituted with one or more different amino acids or (ii) deleted; or (iii) chemically modified.

13. A G-CSF analog of claim 12 wherein said analog is more resistant to proteolysis than a G-CSF molecule of Figure 1.

14 A G-CSF analog of claim 12 wherein at least one of said amino acids is chemically modified by the addition of a polyethylene glycol molecule.

15 15. A G-CSF molecule having the AB loop stabilized by connecting such loop to one or more of helices A, B, C, or D.

16. A G-CSF molecule having the CD loop stabilized by connecting such loop to one or more of helices A, B, C, or D.

20 17. A G-CSF analog, optionally in a pharmaceutically effective carrier, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys¹⁷->Arg¹⁷ and the N-terminal methionine is optional.

25 18. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys³⁵->Arg³⁵ and the N-terminal methionine is optional.

30 19. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys⁴¹->Arg⁴¹ and the N-terminal methionine is optional.

35 20. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that

Lys^{17,24,35}->Arg^{17,24,35} and the N-terminal methionine is optional.

21. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{17,35,41}->Arg^{17,35,41} and the N-terminal methionine is optional.

22. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{24,35,41}->Arg^{24,35,41} and the N-terminal methionine is optional.

23. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{17,24,35,41}->Arg^{17,24,35,41} and the N-terminal methionine is optional.

24. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys^{17,24,41}->Arg^{17,24,41} and the N-terminal methionine is optional.

25. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln⁶⁸->Glu⁶⁸ and the N-terminal methionine is optional.

26. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Cys^{37,43}->Ser^{37,43} and the N-terminal methionine is optional.

27. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln²⁶->Ala²⁶ and the N-terminal methionine is optional.

28. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln¹⁷⁴->Ala¹⁷⁴ and the N-terminal methionine is optional.
29. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg¹⁷⁰->Ala¹⁷⁰ and the N-terminal methionine is optional.
30. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg¹⁶⁷->Ala¹⁶⁷ and the N-terminal methionine is optional.
31. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that there is a deletion at position 167 and the N-terminal methionine is optional.
32. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys⁴¹->Ala⁴¹ and the N-terminal methionine is optional.
33. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that His⁴⁴->Lys⁴⁴ and the N-terminal methionine is optional.
34. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu⁴⁷->Ala⁴⁷ and the N-terminal methionine is optional.
35. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Arg²³->Ala²³ and the N-terminal methionine is optional.

36. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys²⁴->Ala²⁴ and the N-terminal methionine is optional.

5 37. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu²⁰->Ala²⁰ and the N-terminal methionine is optional.

10 38. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Asp²⁸->Ala²⁸ and the N-terminal methionine is optional.

15 39. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met¹²⁷->Glu¹²⁷ and the N-terminal methionine is optional.

20 40. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met¹³⁸->Glu¹³⁸ and the N-terminal methionine is optional.

25 41. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met¹²⁷->Leu¹²⁷ and the N-terminal methionine is optional.

30 42. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met¹³⁸->Leu¹³⁸ and the N-terminal methionine is optional.

35 43. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Cys¹⁸->Ala¹⁸ and the N-terminal methionine is optional.

44. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{12,21}->Glu^{12,21} and the N-terminal methionine is optional.

45. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln^{12,21,68}->Glu^{12,21,68} and the N-terminal methionine is optional.

46. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Glu²⁰->Ala²⁰; Ser¹³->Gly¹³ and the N-terminal methionine is optional.

47. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Met^{127,138}->Leu^{127,138} and the N-terminal methionine is optional.

48. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Ser¹³->Ala¹³ and the N-terminal methionine is optional.

49. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Lys¹⁷->Ala¹⁷ and the N-terminal methionine is optional.

50. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Gln¹²¹->Ala¹²¹ and the N-terminal methionine is optional.

51. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that

Gln²¹->Ala²¹ and the N-terminal methionine is optional.

52. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that
5 His⁴⁴->Ala⁴⁴ and the N-terminal methionine is optional.

53. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein said amino acid sequenc differs from that of Figure 1 in that
His⁵³->Ala⁵³ and the N-terminal methionine is optional.

10 54. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that
Asp¹¹⁰->Ala¹¹⁰ and the N-terminal methionine is optional.

15 55. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that
Asp¹¹³->Ala¹¹³ and the N-terminal methionine is optional.

20 56. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that
Thr¹¹⁷->Ala¹¹⁷ and the N-terminal methionine is optional.

25 57. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that
Asp²⁸->Ala²⁸; Asp¹¹⁰ ->Ala¹¹⁰ and the N-terminal methionine is optional.

30 58. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that
Glu¹²⁴->Ala¹²⁴ and the N-terminal methionine is optional.

59. A G-CSF analog, optionally in a pharmaceutically effective carrier, wherein the amino acid sequence differs from that of Figure 1 in that Phe¹¹⁴->Val¹¹⁴, Thr¹¹⁷->Ala¹¹⁷ and the N-terminal methionine is optional.
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